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## **Interleukins as new prognostic genetic biomarkers in non-small cell lung cancer**

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## 1 TITLE PAGE

### TITLE:

**INTERLEUKINS AS NEW PROGNOSTIC GENETIC BIOMARKERS IN NON-SMALL CELL LUNG CANCER**

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**INTERLEUKINS POLYMORPHISMS IN NSCLC**

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## 2 ABBREVIATIONS LIST

AJCC: American Joint Committee on Cancer

CHUG: Complejo Hospitalario Universitario de Granada

CI: Confidence Interval

EGFR: Epidermal Growth Factor

HR: Hazards Ratio

ILs: Interleukins

NA: Not Available

NSCLC: Non-Small Cell Lung Cancer

OS: Overall Survival

PFS: Progression Free-Survival

SNPs: Single Nucleotide Polymorphisms

## 3 ABSTRACT

### BACKGROUND

Surgery is the standard treatment for early-stage NSCLC, and platinum-based chemotherapy remains as the treatment of choice for advanced-stage NSCLC patients with naïve EGFR status. However, overall 5-years relative survival rates are low. Interleukins (ILs) are crucial for processes associated with tumor development. In NSCLC, *IL1B*, *IL6*, *IL12A*, *IL13* and *IL16* gene polymorphisms may contribute to individual variation in terms of patient survival. The purpose of this study was to evaluate the association between IL gene polymorphisms and survival in NSCLC patients.

### METHODS

A prospective cohorts study was performed, including 170 NSCLC patients (114 Stage IIIB-IV, 56 Stage I-IIIA). *IL1B* (C>T; rs1143634), *IL1B* (C>T; rs12621220), *IL1B* (C>G; rs1143623), *IL1B* (A>G; rs16944), *IL1B* (C>T; rs1143627), *IL6* (C>G; rs1800795), *IL12A* (C>T; rs662959), *IL13* (A>C; rs1881457) and *IL16* (G>T; rs7170924) gene polymorphisms were analyzed by PCR Real-Time.

### RESULTS

Patients with *IL16* rs7170924-GG genotype were in higher risk of death ( $p=0.0139$ ; HR=1.82; CI<sub>95%</sub>=1.13-2.94) Furthermore, carriers of the TT genotype for *IL12A* rs662959 presented higher risk of progression in the non-resected NSCLC patient subgroup ( $p=0.0412$ ; HR=4.49; CI<sub>95%</sub>=1.06-18.99). The rest of polymorphisms showed no effect of on outcomes.

### CONCLUSIONS

Our results suggest that *IL16* rs7170924-GG and *IL12A* rs662959-TT genotypes predict higher risk of death and progression, respectively, in NSCLC patients. No influence of *IL1B* rs12621220, *IL1B* rs1143623, *IL1B* rs16944, *IL1B* rs1143627, *IL6* rs1800795, *IL13* rs1881457 on NSCLC clinical outcomes was found in our patients.

## 4 INTRODUCTION

Lung cancer is the leading cause of death from cancer worldwide, accounting for ≈27% of all cancer deaths. This type of cancer is the second most diagnosed in the United States (after prostate and breast cancer), with an incidence rate over 14% in both genders, and 117920 and 106470 estimated new cases for 2016 in men and women, respectively [1]. In accordance with

the latest cancer statistics, 158080 new cases and 224390 deaths are expected to occur in 2016 [1].

Small cell lung cancer and non-small cell lung cancer (NSCLC) are the two main types of lung cancer. NSCLC represents around 80-85% of all lung cancer cases and is classified in three subtypes: squamous cell carcinoma, adenocarcinoma and large cell carcinoma. According to the American Joint Committee on Cancer (AJCC), most patients with NSCLC present late-stage (IIIB-IV) at the time of diagnosis [2-4].

The standard treatment for early-stage NSCLC is surgery, which may be followed up by platinum-based chemotherapy in patients at high risk of recurrence. Platinum-based chemotherapy remains the treatment of choice for advanced-stage NSCLC. This treatment is given for EGFR (epidermal growth factor receptor) and ALK-rearranged (anaplastic lymphoma kinase) naïve patients and as second line in mutated EGFR patients [5]. Anti-microtubule agents (taxanes and vinca alkaloids), antifolate agents (pemetrexed), or pyrimidine antagonists (gemcitabine) are usually given in combination with cisplatin or carboplatin. In comparison with best supportive care, platinum based chemotherapy has reported more benefits in terms of survival (27.0 vs 10.3 weeks, respectively;  $p < 0.001$ ) and symptom control [6, 7]. However, the overall response rate (ORR) to platinum-based regimen is about 13-47.2% and only 16% of the patients are alive five years after diagnosis [8-24]. Therefore, new therapeutic approaches are urgently needed to improve PFS and OS in advanced stage NSCLC. Pathologic staging is an essential prognostic factor for NSCLC, but a significantly variability in progression and survival among patients with the same stage of disease have been reported, suggesting other factors may influence NSCLC prognosis [4, 25]. Remarkably, genetic alterations, such as single nucleotide polymorphisms (SNPs), have showed to be related with inter-individual differences in recurrence and survival in NSCLC patients [26-29].

Inflammation is a physiological process induced by immune cell to fight infections and heal wounds. Nevertheless, long-standing inflammation secondary to chronic infection may produce a continuous tissue damage and cellular proliferation that results in metaplasia and dysplasia [30, 31]. Therefore, there is a notable association between chronic inflammation, infection and early stage of neoplastic development. In fact, clinical and epidemiological studies have reported that 20% tumors are associated to chronic infection [32]. Interleukins (ILs) are a family of cytokines, which play an essential role on growth, differentiation, and activation of immune cells [33]. Based on above, ILs are crucial for processes associated with tumor development.[34] They act as autocrine and paracrine growth factors, promoting growth and inhibiting apoptosis at the site of inflammation [34]. Remarkably, recent studies have reported a strong effect of *IL1B*, *IL6*, *IL12A*, *IL13* and *IL16* gene polymorphisms on survival of NSCLC patients [26, 27].

IL1B is a pro-inflammatory cytokine that plays a crucial role on inflammatory response, inducing expression of functional genes involved in inflammation [35]. IL1B may be produced by lung epithelial cells and genetic alteration in this gene has showed an important effect on NSCLC development and progression [36-42]. IL6 is a potent pleiotropic inflammatory cytokine that is secreted by lymphoid and non-lymphoid cells, and is involved in important steps of tumor development, such as proliferation, angiogenesis and apoptosis [43-47]. Interestingly, IL6 is expressed in tumor epithelial cells and polymorphisms in this gene have been associated with poor prognosis in NSCLC patients [26]. IL12A is a multifunctional cytokine generated by dendritic cells, macrophages, neutrophils, and human B-lymphoblastoid cells that regulates immune response and induces anti-angiogenesis activity [48]. Germline variations in IL12A decrease its anti-angiogenic effect, resulting in increasing cancer progression [27]. IL13 is a T-cell derived immunoregulatory cytokine produced by T and B cells, mast cells, basophils, natural killer and dendritic cells that exerts a critical function on allergic reactions, inducing immunoglobulin E secretion from activated human B cells [49]. Particularly in cancer, IL13 has reported to be connected with tumor invasion and metastasis by enhancing MAPK pathway [50]. Thus,

polymorphisms in this gene may alter regulation of IL13 production, increasing tumor progression [27]. IL16 is a pro-angiogenic cytokine produced by peripheral blood mononuclear cells that modulate T cell growth [51]. Studies in several types of solid tumors and hematologic malignancies have reported a strong association between IL16 levels and cancer progression [51]. Therefore, polymorphisms in *IL16* gene that reduce angiogenesis process may be responsible for changes in prognosis of NSCLC patients [27].

Based on above, the identification of genetic variants in *ILs* may be essential to predict NSCLC clinical outcomes. To date, there are few studies on germline variations in IL genes and lung cancer survival. In this study, we aimed to evaluate the association between IL gene polymorphisms and survival in NSCLC patients. To determine the impact of treatment, we also performed a subgroup analysis according to surgical resection.

## **5 MATERIAL AND METHODS**

A prospective cohorts study was conducted.

### **5.1 Ethics statement**

This study was performed under the approval of the Complejo Hospitalario Universitario de Granada (CHUG) Ethics and Research Committee and in accordance with the declaration of Helsinki. A written informed consent form was signed by the patients for blood sample collection and genotyping analysis. The identification of samples was based on non-patient codes.

### **5.2 Study population**

This study included 170 NSCLC patients recruited in CHUG, Granada, Spain, diagnosed between 2003-2015 and followed up until February 2016. The inclusion criteria for the group of patients were age  $\geq 18$  years, histologically or cytologically confirmed diagnosis of NSCLC (stages I-IV), an Eastern Cooperative Oncology Group (ECOG) performance status  $\leq 2$ , an adequate organ function, measurable disease by chest computed tomography scan, no previous treatment and available clinical data.

Patients were treated according to the National Comprehensive Cancer Network guidelines [5].

EGFR status was measured by cobas® EGFR Mutation Test [52].

### **5.3 Sociodemographic and clinical variables**

Sociodemographic information including gender, family history of cancer, previous non-lung cancer, previous lung disease, smoking status and age at diagnosis was collected from clinical records. Histopathological data (tumor histology and stage), therapeutic procedure and EGFR status were also collected. Tumors were classified in accordance with the guidelines of the AJCC staging system [53].

### **5.4 Genetic variables**

#### **5.4.1 DNA isolation**

Blood samples (3 ml) were collected in BD Vacutainer® K3E Plus Blood Collection Tubes. Genomic DNA was isolated using the QIAamp DNA Mini Kit (QiagenGmbH, Hilden, Germany) according to the manufacturer's instructions for DNA purification from blood and stored at -40°C.

#### **5.4.2 Detection of gene polymorphisms**

*IL1B* (C>T; rs1143634), *IL1B* (C>T; rs12621220), *IL1B* (C>G; rs1143623), *IL1B* (A>G; rs16944), *IL1B* (C>T; rs1143627), *IL6* (C>G; rs1800795), *IL12A* (C>T; rs662959), *IL13* (A>C; rs1881457) and *IL16* (G>T; rs7170924) gene polymorphisms were analyzed by Real-Time PCR using TaqMan® probes. Genotyping methodology was previously described [54].

### 5.4.3 Survival variables

Survival was measured through OS and PFS. OS was evaluated as time from cancer diagnosis until final follow-up or death and PFS was calculated as the time from initiation of treatment to relapse, death or last known follow-up. Mortality related data were obtained from clinical records and the population-based Cancer Registry of Granada.

### 5.4.4 Statistical Analysis

Quantitative data were expressed as the mean ( $\pm$  standard deviation) for normally-distributed variables or medians and percentiles (25 and 75) for non-normal distributed variables. The Shapiro-Wilks test was used to assess normality.

The Kaplan-Meier method and the log-rank test were used to analyze associations between survival with demographic, clinical and genetic variables. Multivariable Cox proportional hazard regression model (backward stepwise method) was used to obtain the adjusted hazards ratio (HR) and 95% confidence interval (CI<sub>95%</sub>) for potential prognostic factors for survival.

All tests were two-sided with a significance level of  $p < 0.05$ . Data analysis was performed using R 3.0.1 [55].

Hardy Weinberg equilibrium and pairwise haplotype frequencies were estimated using the free, open-source whole genome association analysis toolset PLINK [56].

## 6 RESULTS

### 6.1 Patients characteristics

A total of 170 NSCLC patients were enrolled in the study. The baseline characteristics are summarized in Table 1. Mean age was  $60.46 \pm 10.61$  years, 125 were male (125/170; 73.53%) and 114 were stage IIIB-IV (114/170; 67.06%). Surgery was the first course of treatment for 46 patients (46/170; 27.06%) of which 95.65% (44/46) had stage I-III A and 4.35% (2/46) stage IIIB-IV. During follow-up, 89 death events were recorded. For all patients, median OS and PFS were 32.2 [26.9-52.2] and 15.5 [11.9-18.7] months, respectively.

### 6.2 Influence of clinic-pathologic characteristics on survival

#### 6.2.1 Overall population

Median OS was higher in females ( $p_{\log\text{-rank}} = 0.0183$ ; 52.2 vs 27.0 months; Table S1; Figure S1), squamous cell carcinoma ( $p_{\log\text{-rank}} = 0.0106$ ; 59.4 vs 26.9 months; Table S1; Figure S2), I, II and III A stage ( $p_{\log\text{-rank}} < 0.001$ ; 85.4 vs 24.2 months; Table S1; Figure S3) and surgery as first course of treatment ( $p_{\log\text{-rank}} < 0.001$ ; 114.0 vs 24.5 months; Table S1; Figure S4)

Median PFS was associated with previous lung disease ( $p_{\log\text{-rank}} = 0.0152$ ; 23.2 vs 13.6 months; Table S2; Figure S5), squamous cell carcinoma ( $p_{\log\text{-rank}} = 0.0353$ ; 20.8 vs 15.3 months; Table S2; Figure S6), I, II and III A stage ( $p_{\log\text{-rank}} < 0.001$ ; 48.3 vs 10.9 months; Table S2; Figure S7) and surgery as first course of treatment ( $p_{\log\text{-rank}} < 0.001$ ; 83.9 vs 10.2 months; Table S2; Figure S8)

#### 6.2.2 Subgroup analysis

In the subgroup of resected NSCLC patients, OS and PFS were not associated with clinical or demographic characteristics (Tables S3, S4). However, median OS was higher in female ( $p_{\log\text{-rank}} = 0.012$ ; 47.4 vs 21.4 months; Table S5; Figure S9) and squamous cell carcinoma ( $p_{\log\text{-rank}} = 0.032$ ; 41.8 vs 22.7 months; Table S5; Figure S10) in non-resected NSCLC patients. Median PFS was also associated with gender ( $p_{\log\text{-rank}} = 0.013$ ; 15.8 vs 9.1 months; Table S6; Figure S11) in non-resected NSCLC patients.



### 6.3 Genotypes Distribution

All gene polymorphisms distributions were in agreement with those expected according to the Hardy-Weinberg equilibrium model. Linkage disequilibrium values  $D'$  and  $r^2$  are shown in Table S7. In particular, *IL1B* rs1143627/*IL1B* rs16944 and *IL1B* rs1143623/*IL1B* rs12621220 pairs were in strong linkage disequilibrium.

### 6.4 Influence of gene polymorphisms on survival

#### 6.4.1 Overall population

##### 6.4.1.1 Overall survival

The bivariate analysis showed OS to be associated to *IL16* rs7170924 gene polymorphism (Table S8). In particular, patients with GG genotype were in higher risk of death compared to those carrying the T-allele ( $p=0.0057$ ; HR=1.92; CI<sub>95%</sub>=1.21-3.06; Table S8). Kaplan-Meier curve for OS according to all genotypes and T-allele of *IL16* rs7170924 gene polymorphism is showed in Figure 1A ( $p_{\log\text{-rank}}=0.019$ ) and 1B ( $p_{\log\text{-rank}}=0.005$ ), respectively. Median OS was 27.9 months (CI<sub>95%</sub>=23.9-39.1) for GG genotype. For GT and TT carriers, the median OS was 64.7 (CI<sub>95%</sub>=27.7-not reached [NR]) and 85.6 (CI<sub>95%</sub>=16.0-NR) months, respectively. Multivariate Cox regression adjusted by gender, tumor histology, and first course of treatment showed that *IL16* rs7170924 gene polymorphism was the only independent factor associated to OS ( $p_{\text{likelihood ratio test}}=1.002\cdot 10^{-12}$ ) (Table 2).

##### 6.4.1.2 Progression free-survival

Patients carrying the GG genotype for *IL16* rs7170924 gene polymorphism presented a trend towards higher risk of progression compared to those carrying the CT/CC genotypes, but this was not statistically significant ( $p=0.0739$ ; HR=1.42; CI<sub>95%</sub>=0.97-2.09; Table S9). Kaplan-Meier curves for PFS according to T-allele for *IL16* rs662959 gene polymorphism are showed in Figure S12 ( $p_{\log\text{-rank}}=0.073$ ). Patients with GG genotype showed a median PFS of 13.6 months (CI<sub>95%</sub>=10.2-17.6), whereas for GT and TT genotypes the median PFS was 17.1 (CI<sub>95%</sub>=11.2-82.3) and 25.6 (CI<sub>95%</sub>=10.1-NR) months, respectively.

#### 6.4.2 Subgroup analysis

##### 6.4.2.1 Overall survival

In the resected NSCLC patient subgroup, carriers of the GG genotype for *IL16* rs7170924 gene polymorphism were in higher risk of death compared to those with T-allele ( $p=0.0439$ ; HR=3.50; CI<sub>95%</sub>=1.04-11.85; Table S10). Kaplan-Meier curves for PFS according to T-allele for *IL16* rs662959 gene polymorphism are showed in Figure 2A ( $p_{\log\text{-rank}}=0.034$ ). Median OS for TT carriers was 126.0 (CI<sub>95%</sub>=85.6-NR) months, whereas for GG and GT genotype were not reached. Multivariate Cox regression showed that *IL16* rs7170924 gene polymorphism was the only independent factor associated to OS in resected NSCLC patients ( $p=0.0439$ ; HR=3.50; CI<sub>95%</sub>=1.04-11.85) ( $p_{\text{likelihood ratio test}}=0.03428$ ).

In the subgroup non-resected NSCLC patients, *IL16* rs7170924 gene polymorphism was also associated with higher risk of death ( $p_{\log\text{-rank}}=0.035$ ; Table S11). Figure 2B shows Kaplan-Meier curves for OS according to *IL16* rs662959 gene polymorphism. Median OS for patients carrying TT genotype was 16.0 (CI<sub>95%</sub>=5.6-NR) months, whereas for GG and GT genotypes was 23.2 (CI<sub>95%</sub>=18.3-30.7) and 27.7 (CI<sub>95%</sub>=22.7-NR) months, respectively.

##### 6.4.2.2 Progression free-survival

In the resected NSCLC subgroup, patients carrying the TT genotype for *IL1B* rs1143634 gene polymorphism showed a trend towards higher progression, compared to those with C-allele, but this was not statistically significant ( $p=0.104$ ; HR=5.69; CI<sub>95%</sub>=0.70-46.28; Table S12). Kaplan-Meier curves for PFS according to C-allele for *IL1B* rs1143634 gene polymorphism are showed

in Figure S13 ( $p_{\log\text{-rank}}=0.064$ ). Patients with TT genotype showed a median PFS of 15.3 months ( $CI_{95\%}=\text{NR-NR}$ ), whereas for CC genotype was 54.3 ( $CI_{95\%}=41.9\text{-NR}$ ), and for CT was not reached.

*IL12A* rs662959 gene polymorphism showed influence on PFS only in non-resected NSCLC patients (Table S13). In fact, patients carrying the TT genotype presented higher risk of progression compared to those carrying the CT/CC genotypes ( $p=0.0332$ ;  $HR=4.77$ ;  $CI_{95\%}=1.13\text{-}20.07$ ; Table S13). Kaplan-Meier curves for PFS according to all genotypes and C-allele for *IL12A* rs662959 gene polymorphism are showed in Figure 3A ( $p_{\log\text{-rank}}=0.031$ ) and 3B ( $p_{\log\text{-rank}}=0.018$ ), respectively. Patients with TT genotype showed a median PFS of 4.3 months ( $CI_{95\%}=3.7\text{-NR}$ ), whereas for CC and CT genotypes, the median PFS was 10.2 ( $CI_{95\%}=8.4\text{-}13.2$ ) and 15.0 ( $CI_{95\%}=6.0\text{-NR}$ ) months, respectively. A multivariate Cox regression model adjusted by gender and tumor histology was used to evaluate the impact of *IL12A* rs662959 gene polymorphism on PFS (Table 3). *IL12A* rs662959 gene polymorphism remained significantly associated with PFS ( $p_{\text{likelihood ratio test}}=0.001736$ ). Furthermore, non-resected NSCLC patients carrying the A-allele for *IL1B* rs16944 gene polymorphism showed a trend towards higher risk of progression compared to those with GG genotype, but this was not statistically significant ( $p=0.0877$ ;  $HR=1.45$ ;  $CI_{95\%}=0.95\text{-}2.22$ ; Table S13). Kaplan-Meier curves for PFS according to A-allele for *IL1B* rs16944 gene polymorphism are showed in Figure S14 ( $p_{\log\text{-rank}}=0.0806$ ). Median PFS for carriers of *IL1B* rs16944-AA genotype was 7.5 ( $CI_{95\%}=4.7\text{-NR}$ ), whereas for AG and GG genotype was 10.0 ( $CI_{95\%}=6.5\text{-}12.9$ ) and 15.0 ( $CI_{95\%}=10.0\text{-}19.6$ ) months, respectively. Similarly, the C-allele of *IL1B* rs1143627 gene polymorphisms, which was in linkage disequilibrium with *IL1B* rs16944, presented a trend towards higher risk of progression in non-resected NSCLC subgroup, but this was not statistically significant either ( $p=0.077$ ;  $HR=1.45$ ;  $CI_{95\%}=0.45\text{-}1.06$ ; Table S13). Kaplan-Meier curves for PFS according to C-allele for *IL1B* rs1143627 gene polymorphism are showed in Figure S15 ( $p_{\log\text{-rank}}=0.086$ ; Table S13). Patients with CC genotype showed a median PFS of 8.0 months ( $CI_{95\%}=4.7\text{-NR}$ ), whereas for CT and TT genotypes the median PFS was 9.1 ( $CI_{95\%}=6.5\text{-}14.6$ ) and 15.0 ( $CI_{95\%}=10.0\text{-}19.6$ ) months, respectively.

## 7 DISCUSSION

Surgery is the standard treatment for early-stage NSCLC and platinum-based chemotherapy remains as the treatment of choice for advanced-stage NSCLC patients with naïve EGFR status. However, overall 5-years relative survival rates are low, with great inter-individual differences, which may be largely explained due to genetic factors. Several polymorphisms in different genes involved in inflammatory response have been proposed as potential causes of this variability [57].

In this study, 170 not previously treated NSCLC patients from a single institution were enrolled to investigate the potential role of *IL1B*, *IL6*, *IL12*, *IL13* and *IL16* gene polymorphisms in clinical outcomes. In the overall population, the GG genotype for *IL16* rs7170924 showed to be a poor prognosis factor (higher risk of death) (Table 2), in agreement with a previous study, which showed worse PFS in 651 Caucasian patients (stage I-IV) for those carrying the *IL16*-GG genotype ( $HR=0.65$ ;  $CI_{95\%}=0.50, 0.83$  for GT/TT vs GG) [27]. We also found that in non-resected NSCLC patients, carriers of the TT genotype for *IL12A* rs662959 presented higher risk of progression compared to those carrying the CT/CC genotypes, in agreement with a previous study ( $HR=1.41$ ;  $CI_{95\%}=1.08, 1.83$  for CT/TT vs CC) [27]. To date, no other studies have explored the association between *IL16* or *IL12A* and NSCLC outcomes. However, other polymorphisms in *IL16* (rs4778889, rs11556218, rs1131445) and *IL12A* (rs568408, rs3212227, rs3181224) have reported association with risk of renal cell cancer, glioma, gastric cancer, lung cancer, nasopharyngeal cancer, hepatocellular carcinoma, cervical and vulvar cancer [39, 58-62].

The mechanism by which *IL16* and *IL12* influence prognosis in NSCLC is still unknown. Several studies have associated altered serum ILs levels with greater susceptibility to cancer development and progression [63-66]. In colorectal cancer, it has been demonstrated that *IL6* is

overexpressed and may regulate CYP1B1 expression via miR27b [67]. CYP1B1 activates many pro-cancer substances, such as heterocyclic amines and polycyclic aromatic hydrocarbons [68]. Thus, it is possible that IL16 and IL12 may regulate cytochrome P450 enzyme expression via microRNA and subsequently promote pro-carcinogen activation and DNA damage. In addition, cytokines promote a neoplastic phenotype through the activation of a variety of signal pathways that are involved in cell survival, invasion, adhesion, proliferation and migration such as JAK/STAT, PTEN/PI3K/AKT and RAS/RAF/MEK/ERK cascade signaling [69, 70]. Therefore, the promotion of these hallmark cancer pathways may contribute to the development of platinum-based chemotherapy resistance. Based on above, IL16 and IL12A levels may play a crucial role on NSCLC prognosis and drug resistance.

On the other hand, genetic factors may influence production and/or activity of cytokines, causing variations which may influence on cancer prognosis [71-73]. The effect of *IL16* rs7170924 and *IL12A* rs662959 polymorphisms on cytokine levels is still unknown. However, a potential alteration of cytokine secretion by *IL16* rs7170924 and *IL12A* rs662959 polymorphisms may underlie the strong effect on survival observed in NSCLC patients. In our study, no serum IL1B, IL6, IL12A, IL13, IL16 levels assessment was determined, therefore we cannot establish if they were altered in patients with *IL16* rs7170924-GG and *IL12A* rs662959-TT genotypes.

A recent study in 651 Caucasian stage I-IV NSCLC patients reported better OS (HR=0.78; 95%CI=0.63, 0.98 for CT/TT vs CC) and PFS (HR=0.73; CI<sub>95%</sub>=0.57, 0.93 for CT/TT vs CC) for *IL1B* rs1143634 [27]. In our patients, this SNP, along with *IL1B* rs12621220, rs1143623, rs16944 and rs1143627, were not associated with clinical outcomes. However, a trend towards higher risk of progression was found for *IL1B* rs1143634-TT genotype in resected NSCLC patients, and *IL1B* rs16944-A and *IL1B* rs1143627-C alleles in non-resected NSCLC patients (not statistically significant). To date, these polymorphisms have not been associated to NSCLC outcomes, despite they have been related to lung cancer susceptibility [41]. *IL6* rs1800795 was not associated either with survival in our patients, despite a previous study with 434 Caucasian stage I-IV patients reported that the *IL6* rs1800795 G-allele was associated with poor OS [74]. Although the C-allele of rs1881457 polymorphism in *IL13* predicted higher risk of progression (HR=1.29; CI<sub>95%</sub>=1.00, 1.66 for AC/CC vs AA) in 651 Caucasian stage I-IV NSCLC patients, this effect was not confirmed in our patients [27].

This study presents a cohort of NSCLC patients recruited from the same hospital and treated under the same protocols and guidelines, which ensures a homogeneous sample regarding treatment administered and measure of survival variables. Although the size of the sample was limited, and some associations may have been underpowered to be detected, the effect of *IL16* and *IL12A* was evident.

In summary, these results suggested that *IL16* rs7170924 and *IL12A* rs662959 polymorphisms may substantially act as prognostic factors in NSCLC patients.

## 8 CONCLUSIONS

Our results suggest that *IL16* rs7170924-GG and *IL12A* rs662959-TT genotypes predict higher risk of death and progression, respectively, in NSCLC patients. No influence of *IL1B* rs12621220, *IL1B* rs1143623, *IL1B* rs16944, *IL1B* rs1143627, *IL6* rs1800795, *IL13* rs1881457 on NSCLC clinical outcomes was found in our patients.

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## 10 CONFLICT OF INTEREST

The authors declare that there is not conflict of interest that could be perceived as prejudicing the impartiality of the research reported and there is not any competing financial interest in relation to the work described in this article.

## 11 FIGURE LEGENDS

Figure 1. (A) Kaplan-Meier curve for overall survival according to all genotypes of IL16 rs7170924 gene polymorphism in 170 NSCLC patients. (B) Kaplan-Meier curve for overall survival according to T-allele of IL16 rs7170924 gene polymorphism in 170 NSCLC patients.

Figure 2. (A) Kaplan-Meier curve for overall survival according to T-allele of IL16 rs7170924 gene polymorphism in the resected NSCLC subgroup. (B) Kaplan-Meier curve for overall survival according to all genotypes of IL16 rs7170924 gene polymorphism in the non-resected subgroup.

Figure 3. (A) Kaplan-Meier curve for progression-free survival according to all genotypes of IL12A rs662959 gene polymorphism in the non-resected NSCLC subgroup.(B) Kaplan-Meier curve for progression-free survival according to C-allele of IL12A rs662959 gene polymorphism in the non-resected NSCLC subgroup.

Figure S1. Kaplan-Meier curve for overall survival according to gender in 170 NSCLC patients.

Figure S2. Kaplan-Meier curve for overall survival according to histology in 170 NSCLC patients.

Figure S3. Kaplan-Meier curve for overall survival according to tumor stage in 170 NSCLC patients.

Figure S4. Kaplan-Meier curve for overall survival according to first course of treatment (divided by surgery) in 170 NSCLC patients.

Figure S5. Kaplan-Meier curve for progression-free survival according to previous lung disease in 170 NSCLC patients.

Figure S6. Kaplan-Meier curve for progression-free survival according to histology in 170 NSCLC patients.

Figure S7. Kaplan-Meier curve for progression-free survival according to tumor stage in 170 NSCLC patients.

Figure S8. Kaplan-Meier curve for progression-free survival according to first course of treatment (divided by surgery) in 170 NSCLC patients.

Figure S9. Kaplan-Meier curve for overall survival according to gender in the non-resected NSCLC subgroup.

Figure S10. Kaplan-Meier curve for overall survival according to histology in the non-resected NSCLC subgroup.

Figure S11. Kaplan-Meier curve for progression-free survival according to gender in the non-resected NSCLC subgroup.

Figure S12. Kaplan-Meier curve for progression-free survival according to T-allele of IL16 rs7170924 gene polymorphism in 170 NSCLC patients.

Figure S13. Kaplan-Meier curve for progression-free survival according to C-allele of IL1B rs1143634 gene polymorphism in the resected NSCLC subgroup.

Figure S14. Kaplan-Meier curve for progression-free survival according to A-allele of IL1B rs16944 gene polymorphism in the non-resected NSCLC subgroup.

Figure S15. Kaplan-Meier curve for progression-free survival according to C-allele of IL1B rs1143627 gene polymorphism in 170 NSCLC patients.

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